

in other tissues may be due to the presence of macrophages and dendritic cells, in which it could act as an early-warning system for infection. The public GenBank database contains the following Toll sequences: Toll1 (DNAX# HSU88540-1, which is identical with the random sequenced full-length cDNA #HUMRSC786-1); Toll2 (DNAX# HSU88878-1); Toll3 (DNAX# HSU88879-1); and Toll4 (DNAX# HSU88880-1, which is identical with the DNA sequence reported by Medzhitov *et al.*, *supra*). A partial Toll sequence (Toll5) is available from GenBank under DNAX# HSU88881-1.

Further human homologues of the *Drosophila* Toll protein, designated as Toll-like receptors (huTLRs1-5) were recently cloned and shown to mirror the topographic structure of the *Drosophila* counterpart (Rock *et al.*, *Proc. Natl. Acad. Sci. USA* **95**, 588-593 [1998]). Overexpression of a constitutively active mutant of one human TLR (Toll-protein homologue - Medzhitov *et al.*, *supra*; TLR4 - Rock *et al.*, *supra*) leads to the activation of NF- $\kappa$ B and induction of the inflammatory cytokines and constimulatory molecules. Medzhitov *et al.*, *supra*.

#### 84. PRO213-1, PRO1330 and PRO1449

Cancer is characterized by the increase in the number of abnormal, or neoplastic, cells derived from a normal tissue which proliferate to form a tumor mass, the invasion of adjacent tissues by these neoplastic tumor cells, and the generation of malignant cells which eventually spread via the blood or lymphatic system to regional lymph nodes and to distant sites (metastasis). In a cancerous state a cell proliferates under conditions in which normal cells would not grow. Cancer manifests itself in a wide variety of forms, characterized by different degrees of invasiveness and aggressiveness.

Alteration of gene expression is intimately related to the uncontrolled cell growth and de-differentiation which are a common feature of all cancers. The genomes of certain well studied tumors have been found to show decreased expression of recessive genes, usually referred to as tumor suppression genes, which would normally function to prevent malignant cell growth, and/or overexpression of certain dominant genes, such as oncogenes, that act to promote malignant growth. Each of these genetic changes appears to be responsible for importing some of the traits that, in aggregate, represent the full neoplastic phenotype (Hunter, *Cell* **64**, 1129 [1991]; Bishop, *Cell* **64**, 235-248 [1991]).

A well known mechanism of gene (e.g. oncogene) overexpression in cancer cells is gene amplification. This is a process where in the chromosome of the ancestral cell multiple copies of a particular gene are produced. The process involves unscheduled replication of the region of chromosome comprising the gene, followed by recombination of the replicated segments back into the chromosome (Alitalo *et al.*, *Adv. Cancer Res.* **47**, 235-281 [1986]). It is believed that the overexpression of the gene parallels gene amplification, i.e. is proportionate to the number of copies made.

Proto-oncogenes that encode growth factors and growth factor receptors have been identified to play important roles in the pathogenesis of various human malignancies, including breast cancer. For example, it has been found that the human *ErbB2* gene (*erbB2*, also known as *her2*, or *c-erbB-2*), which encodes a 185-kd transmembrane glycoprotein receptor (p185HER2; HER2) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon *et al.*, *Science* **235**:177-182 [1987]; Slamon *et al.*, *Science* **244**:707-712 [1989]).

It has been reported that gene amplification of a protooncogene is an event typically involved in the more malignant forms of cancer, and could act as a predictor of clinical outcome (Schwab et al., *Genes Chromosomes Cancer* 1, 181-193 [1990]; Alitalo et al., *supra*). Thus, erbB2 overexpression is commonly regarded as a predictor of a poor prognosis, especially in patients with primary disease that involves axillary lymph nodes (Slamon et al., [1987] and [1989], *supra*; Ravdin and Chamness, *Gene* 159:19-27 [1995]; and Hynes and Stern, *Biochem Biophys Acta* 1198: 165-184 [1994]), and has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluoruracil) and anthracyclines (Baselga et al., *Oncology* 11 (3 Suppl 1): 43-48 [1997]). However, despite the association of erbB2 overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to treatment with taxanes were greater than three times those of HER2-negative patients (Ibid). A recombinant humanized anti-ErbB2 (anti-HER2) monoclonal antibody (a humanized version of the murine anti-ErbB2 antibody 4D5, referred to as rhuMAb HER2 or Herceptin 78) has been clinically active in patients with ErbB2-overexpressing metastatic breast cancers that had received extensive prior anticancer therapy. (Baselga et al., *J. Clin. Oncol.* 14:737-744 [1996]).

The protein Notch and its homologues are key regulatory receptors in determining the cell fate in various development processes. The protein Notch-4, also known as int-3 oncogene, was originally identified as a frequent target in mouse mammary tumor virus (MMVS). Notch-4 is believed to be a transgene which affects the differentiation capacity of stem cells and leads to neoplastic proliferation in epithelial cells. Shirayoshi et al., *Genes Cells* 2(3): 213-224 (1997). During embryogenesis, the expression of Notch-4 was detected in endothelial cells of blood vessels forming tissues such as the dorsal aorta, intersegmental vessels, yolk sac vessels, cephalic vessels, heart, vessels in branchial arches, and capillary plexuses. Notch-4 expression in these tissues was also associated with flk-1, the major regulatory gene of vasculogenesis and angiogenesis. Notch-4 is also upregulated in vitro during the differentiation of endothelial stem cell. The endothelial cell specific expression pattern of Notch-4, as well as its structural similarity to Notch suggest that Notch-4 is an endothelial cell specific homologue of Notch and that it may play a role in vaculogenesis and angiogenesis.

#### 85. PRO298

Efforts are being undertaken by both industry and academia to identify new, native receptor proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel receptor proteins. We herein describe the identification and characterization of novel transmembrane polypeptides, designated herein as PRO298 polypeptides.

#### 86. PRO337

Neuronal development in higher vertebrates is characterized by processes that must successfully navigate distinct cellular environment en route to their synaptic targets. The result is a functionally precise formation of neural circuits. The precision is believed to result from mechanisms that regulate growth cone pathfinding and target recognition, followed by latter refinement and remodeling of such projections by events that require neuronal activity, Goodman and Shatz, *Cell/Neuron [Suppl.]* 72(10): 77-98 (1993). It is further evident that different neurons extend nerve fibers that are biochemically distinct and rely on specific guidance cues provided

by cell-cell, cell-matrix, and chemotrophic interactions to reach their appropriate synaptic targets, Goodman et al., *supra*.

One particular means by which diversity of the neuronal cell surface may be generated is through differential expression of cell surface proteins referred to as cell adhesion molecules (CAMs). Neuronally expressed CAMs have been implicated in diverse developmental processes, including migration of neurons along radial glial cells, providing permissive or repulsive substrates for neurite extension, and in promoting the selective fasciculation of axons in projectional pathways. Jessel, *Neuron* 1: 3-13 (1988); Edelman and Crossin, *Annu. Rev. Biochem.* 60: 155-190 (1991). Interactions between CAMs present on the growth cone membrane and molecules on opposing cell membranes or in the extracellular matrix are thought to provide the specific guidance cues that direct nerve fiber outgrowth along appropriate projectional pathways. Such interactions are likely to result in the activation of various second messenger systems within the growth cone that regulate neurite outgrowth. Doherty and Walsh, *Curr. Opin Neurobiol.* 2: 595-601 (1992).

In higher vertebrates, most neural CAMs have been found to be members of three major structural families of proteins: the integrins, the cadherins, and the immunoglobulin gene superfamily (IgSF). Jessel, *supra.*; Takeichi, *Annu. Rev. Biochem.* 59: 237-252 (1990); Reichardt and Tomaselli, *Annu. Rev. Neurosci.* 14: 531-570 (1991). Cell adhesion molecules of the IgSF (or Ig-CAMs), in particular, constitute a large family of proteins frequently implicated in neural cell interactions and nerve fiber outgrowth during development, Salzer and Colman, *Dev. Neurosci.* 11: 377-390 (1989); Brümmendorf and Rathjen, *J. Neurochem.* 61: 1207-1219 (1993). However, the majority of mammalian Ig-CAMs appear to be too widely expressed to specify navigational pathways or synaptic targets suggesting that other CAMs, yet to be identified, have role in these more selective interactions of neurons.

Many of the known neural Ig-CAMs have been found to be attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor. Additionally, many studies have implicated GPI-anchored proteins in providing specific guidance cues during the outgrowth on neurons in specific pathways. In studies of the grasshopper nervous system, treatment of embryos with phosphatidylinositol-specific phospholipase C (PIPLC), which selectively removes GPI-anchored proteins from the surfaces of cells, resulted in misdirection and faulty navigation among subsets of pioneering growth cones, as well as inhibited migratory patterns of a subset of early neurons, Chang et al., *Devel.* 114: 507-519 (1992). The projection of retinal fibers to the optic tectum appears to depend, in part, on a 33 kDa GPI-anchored protein, however, the precise nature of this protein is unknown. Stahl et al., *Neuron* 5: 735-743 (1990).

The expression of various GPI-anchored proteins has been characterized amongst the different populations of primary rat neurons amongst dorsal root ganglion, sympathetic neurons of the cervical ganglion, sympathetic neurons of the superior cervical ganglion, and cerebellar granule neurons. Rosen et al., *J. Cell Biol.* 117: 617-627 (1992). In contrast to the similar pattern of total membrane protein expression by these different types of neurons, striking differences were observed in the expression of GPI-anchored proteins between these neurons. Recently, a 65 kDa protein band known as neurotrimin was discovered and found to be differentially expressed by primary neurons (Rosen et al., *supra*), and restricted to the nervous system and found to be the most abundant and earliest expressed of the GPI-anchored species in the CNS. Struyk et al., *J. Neuroscience* 15(3): 2141-2156 (1995). The discovery of neurotrimin has further lead to the identification of a family of IgSF